Lab Notes

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TO : File

FROM : Joshua Lederberg

SUBJECT: Biology - Notes

Grace remarked that her experiments on trying to time the synthesis of different genes by extracting DNA after spore germination do not work. She indicated that it appeared that only thymine requiring strains work by this procedure and that Gan believed that phage induction was involved. She is planning to introduce the thymine-negative marker into her strains in order to pursue the experiment.

Well, why not try other inducing agents. This would simultaneously test Gan's model and provide a convenient way to do experiments of this kind. We had in mind ultraviolet light and mitomicin.

Betty Cohen discussed her results with what looks like lysozyme killing of her organism. She remarked that she is able to get 95% killing of non-competent cells just by going through the heat shock procedure, and then remarked that in other experiments, which had not been heat shocked, she had had 100% survival. Putting these together led to a recollection of the reports from Westphal's laboratory on the effect of temperature on mobilization of surface LPS and a more comprehensive general model along the following lines. Perhaps it is the heat shock which is instrumental in stripping the cells of an LPS and sensitizing them to lysozyme. The cells must then be chilled to prevent them from resynthesizing or remobilizing the LPS. Our previous efforts to increase transformation or transfection by the use of lysozyme were therefore doomed to failure. It is sensitivity to lysozyme, not response to it, which is the critical parameter. So, in effect response to lysozyme is a signature of responsivity to DNA.

Is lysozyme an acidic protein and is this its common feature with DNA?

It is a little difficult to reconstruct all of the details that had been put into this analysis before that abductive leap. That so simplified the interpretation that it is now dofficult to recreate our former pattern of confusion. However, the crucial ingredient is bringing in the notion that chilling is necessary in order to prevent the recovery of the cells from the previous shock.

Another abductive suggestion is to look for protoplast formation as an alternative to lysozyme killing on sensitive cells. Ron talked about the kinetics of inactivation. He has been following the movement of various markers from the heavy DNA into specific lighter segments as a function of time and finds that this is variable for different markers. One abductive question was whether he had in fact tested that distribution at zero minutes and in fact he had neglected to do so.

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In his studies on the kinetics of inactivation he has rediscovered the early fall and partial recovery that Frank Young had mentioned to me. Who then proceeded to analyze possible hypotheses fairly systematically. They fell into the following categories:

(a)Reannealing of segmented structures. This was also Frank Young's idea but it is not obvious why this should follow that particular kinetic picture.

(b) The existence of nicked structures which are not totally sliced which potentially may be less active either than the larger DNA to start with or the smaller segments later on. The way to study this phenomenon is (1) to attempt to isolate the structures, see what they look like, see what the consequences of further treatment with R1 will be, or (2) to use ligase to heal the nicks and see if this restores activity. The nicking might be the consequence either of incomplete action of R1 or of contaminating enzyme in the R1.

(c) Ron pointed out that R1 might be reversible or might have side-effects or might be contaminated, and these are paradigms that certainly should be remembered in every circumstance. The reversibility of R1 effects is a little perplexing to put into the immediate picture.

(d) One should remember that R1 is a protein that binds to the enzyme and these enzyme DNA complexes might have peculiar activity.As a subheading on this, perhaps R1 looks sequentially at 2 sites that it might be a two-headed enzyme and this immediately brings up the picture of providing linkage between two disparate DNA molecules perhaps giving inactive structures. This is reminiscent of last week's discussion where we also discussed the possibility of a tightly bound protein inhibitor that can be removed by phenol extraction that has not been looked into.

Ron also pointed out that there are different rates of equilibration or digestion. It is certain that metB5 is not fully digested at a time when tryp seems to be asymptotic. Why perhaps are there different rates - the abductions are:(1)the DNA may be isolated in a form where some sites have bound protein in contrast to others. However, this has already been partially tested with phenol extraction and that does not seem to matter. (2) There might be inherent differences in susceptibility for different DNA sequences. The plausibility of this is augmented by the fact that this has already been demonstrated in lambda that there are different rates of digestion of different parts. Further abduction, this would be much more readily tested in B. subtilis when we have homogenous amplified segments containing restriction sites for a different battery of enzymes.

These findings with some slight residual activity even after limit digestion suggest again the search for site resistant mutants. The protocol for this is fairly obvious although it can be expanded by recycling the selection for the survivors of the DNA treatment,and the same paradigm in fact can be used for the selection for co-transfer which is rather low in some rather large pieces.

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A further lemma from this is that selection for site resistance might also select for deletion and that one should take precautions like looking for or at least being able to cover mutations at other sites in selecting for these events.

The linkage story was that even homogenous segments in the ILV region show very, very low linkage, I believe between ILV and lysine. Since the DNA is homogenous, we cannot use the usual excuses of different sized fragments. However, nicks might be there abduction test some kind of alkaline gradient methodology for this purpose. Again, this would be much more easy to do with amplified segments.

Some take-home messages: (1) be sure to look up Repaski's work on lysozyme and
(2) to check whether lysozyme is an acid protein; abduction can one generalize as to which proteins do require the stripping of surface sites for entry and which do not.

Another more general take-home lesson - it will save a lot of time to consult experts like Gan with respect to thymine action or the literature with respect to lysozyme before committing a lot of effort. I pointed out that our actual gross costs of running the lab are about $2,000 a week and one should keep this in mind in trying to enhance the efficiency of da-to-day lab operations and especially not to waste time on experiments that might be avoided or that one could prevent from needless repetition.